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Regulation of plasma leptin in mice: influence of age, high-fat diet, and fasting

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Departments of [1]Medicine and [2]Radiology, Lund University, Malmö, Sweden S-205 02; [3]Linco Research, St. Charles, Missouri 63301; and [4]Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, and Department of Nutrition, University of California, Davis, California 95616

Ahrén, Bo, Sven Månsson, Ronald L. Gingerich, and Peter J. Havel. Regulation of plasma leptin in mice: influence of age, high-fat diet, and fasting. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R113–R120, 1997.—Mechanisms regulating circulating leptin are incompletely understood. We developed a radioimmunoassay for mouse leptin to examine the influence of age, dietary fat content, and fasting on plasma concentrations of leptin in the background strain for the *ob/ob* mouse, the C57BL/6J mouse. Plasma leptin increased with age [5.3 ± 0.6 ng/ml at 2 mo (n = 23) vs. 14.2 ± 1.6 ng/ml at 11 mo (n = 15), $P < 0.001$]. Across all age groups (2–11 mo, n = 160), log plasma leptin correlated with body weight (r = 0.68, $P < 0.0001$), plasma insulin (r = 0.38, $P < 0.001$), and amount of intra-abdominal fat (r = 0.90, $P < 0.001$), as revealed by magnetic resonance imaging. Plasma leptin was increased by a high-fat diet (58% fat for 10 mo) and reduced by fasting for 48 h. The reduction of plasma leptin was correlated with the reduction of plasma insulin (r = 0.43, P = 0.012) but not with the initial body weight or the change in body weight. Moreover, the reduction in plasma leptin by fasting was impaired by high-fat diet. Thus plasma leptin in C57BL/6J mice *1*) increases with age or a high-fat diet; *2*) correlates with body weight, fat content, and plasma insulin; and *3*) is reduced during fasting by an action inhibited by high-fat diet and related to changes of plasma insulin.

ob gene; insulin; C57BL/6J

LEPTIN, a 16-kDa protein, is the product of the *ob* gene (32). Previous studies in humans and rodents have revealed that the expression of the *ob* gene correlates with body fat content (11, 20, 22). After the development of radioimmunoassay for leptin, it has been demonstrated that body fat content also correlates with circulating plasma leptin levels (7–9, 16, 18, 19, 22, 23). In addition, leptin administration has been shown to reduce food intake and increase the energy expenditure in mice (4, 12, 26). It has therefore been hypothesized that leptin is a humoral signal from adipose tissue that acts on the central nervous system to reduce food intake in a negative feedback manner (4, 5, 22, 26). Thus circulating leptin may be a regulator of body adiposity, and therefore factors that influence the nutritional status might operate through changes of circulating leptin. However, the factors involved in the regulation of circulating leptin concentrations are incompletely understood. Therefore, we have developed a radioimmunoassay specific for mouse leptin to examine the changes in plasma leptin in C57BL/6J mice, the background strain for the *ob/ob* mouse, during aging as well as after treatment with high-fat diet, which increases body weight and induces insulin resistance and *ob* gene expression in this strain (6, 24, 29), and after fasting, which reduces body weight and lowers plasma insulin. We also characterized the relationship between plasma leptin and both intra-abdominal and subcutaneous fat content as revealed by magnetic resonance imaging (MRI), a sensitive technique for quantifying body fat content (1).

The study in particular examined the relationship between plasma leptin and plasma insulin during aging and after high-fat diet and fasting, because it has previously been demonstrated that increased body fat content, as seen in obesity, is accompanied by insulin resistance that induces hyperinsulinemia (25) and that insulin increases *ob* gene expression in human, rat, or mouse adipocytes (8, 21, 24, 27, 31). Therefore, it is possible that insulin contributes to increased *ob* gene expression and the elevated circulating leptin seen in obesity. Regulation of *ob* gene expression by insulin is also supported by findings that *ob* gene expression is reduced in streptozotocin-induced diabetes, which is accompanied by hypoinsulinemia (3, 21, 28). Similarly, fasting, which reduces circulating insulin, lowers both expression of *ob* gene (3, 8, 11, 21, 28) and circulating leptin (2, 11, 22). However, regulation of *ob* gene expression by insulin has been questioned in two studies showing that administration of insulin does not normalize the lowered *ob* gene expression seen in streptozotocin-induced diabetes (3, 28), although one study reported normalization by insulin (21). Moreover, in humans, short-term administration of moderate doses of insulin does not seem to alter plasma leptin (9, 18, 19), whereas supraphysiological hyperinsulinemia (>4 h) does increase plasma leptin in both normal and diabetic subjects (14, 30). Therefore, the relationship between plasma leptin and physiological changes in plasma insulin is not established and needs to be further explored.

MATERIALS AND METHODS

Animals. Mice of the C57BL/6J strain were obtained from Bomholtgaard Breeding and Research Centre, Ry, Denmark, at 4 wk of age. Only female mice were used to avoid the profound gender differences in circulating leptin that have been documented in humans (15). One-half of the mice in each batch received a high-fat diet (Research Diets, New Brunswick, NJ), and the other one-half of the animals received an ordinary rodent chow diet (Lactamin, Stockholm, Sweden). On a caloric basis, the high-fat diet consisted of 16.4% protein, 25.6% carbohydrate, and 58.0% fat (total 23.4 kJ/g), and the control diet consisted of 25.8% protein, 62.8% carbohydrate, and 11.4% fat (total 12.6 kJ/g). Throughout the

study period, the mice had free access to food and water. Four to five mice were kept per cage in a temperature-controlled (22 ± 1°C) room with a 12:12-h light-dark cycle with light on at 0600. The study was approved by the Animal Ethics Committee at Lund University.

Experiments. After 1, 2, 6, and 10 mo of treatment with high-fat diet or control diet, i.e., at the age of 2, 3, 7, and 11 mo, the animals were weighed, and a blood sample taken from the intraorbital retrobulbar plexus was placed in heparinized, prechilled tubes for the measurement of plasma levels of leptin, insulin, and glucose. Furthermore, in the two groups, at 3 mo of age, food but not water was withdrawn from the cages for 48 h, after which an additional blood sample was taken and the animals were weighed. Plasma was immediately separated after centrifugation at 4°C and stored at −20°C until analysis.

Analyses. A new radioimmunoassay (Linco Research, St. Charles, MO) was developed to measure mouse leptin in plasma or serum using a polyclonal antibody raised in rabbits against highly purified recombinant mouse leptin. Calibrators (0.2, 0.5, 1.0, 2.0, 5.0, 1.0, and 20.0 ng/ml) and ^{125}I-labeled tracer were prepared with recombinant mouse leptin. Calibrators (100 μl) or specimens (25–100 μl) in duplicate were mixed with antibody (100 μl) and incubated overnight at 4°C. If specimen volume was <100 μl, the remaining volume was adjusted with buffer. ^{125}I-leptin (100 μl) was added, mixed, and incubated an additional 24 h at 4°C. One milliliter of precipitating reagent [anti-rabbit rabbit immunoglobin G (IgG)] was added to all tubes (except totals) and incubated for 20 min at 4°C to precipitate the antibody-antigen complex. Tubes were centrifuged for 15 min at 2,000 *g* at 4°C. The supernatants were decanted and the pellets were counted to determine bound activity. Calculation of unknown concentrations was accomplished by log/logit transformation. Recovery of different amounts of mouse leptin added to a mouse serum pool (starting leptin 0.4 ng/ml) is shown in Table 1. Recovery ranged from 92 to 100% over a range of 0.4–5.1 ng/ml. Linear dilution of four mouse serum specimens (initial concentrations 2.2, 4.6, 8.1, and 10.5 ng/ml) is shown in Table 2. Specimens were measured at 100, 75, and 50 μl of serum. Recoveries ranged from 100 to 125%. Within- and between-assay variations were assessed by repeated analysis of four serum samples containing 0.4–5.4 ng/ml leptin. Coefficients of variations (CV) ranged from 4.0 to 11.2% within runs and from 3.3 to 14.6% between runs (Table 3). Plasma insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, ^{125}I-labeled porcine insulin as tracer, and rat insulin as standard (Linco). Free and bound radioactivity were separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l, and the CV is <7% at both low and high levels. Glucose was determined with the glucose oxidase method.

MRI. After 10 mo on high-fat or control diet, six mice from each group underwent an MRI examination. All images were obtained on an animal research MRI system with a magnetic field strength of 2.4 tesla (Bruker Biospec 24/30, Karlsruhe, Germany). T1-weighted spin echo images were acquired in the axial plane with the following parameters: repetition time/echo time = 150/5 ms; matrix size, 192 × 128; field of view, 5 × 4 cm. Five contiguous slices of 3-mm thickness and a total imaging time of 4 min were performed in each animal. Before the imaging, the nonfasted animals were anesthetized with an intraperitoneal injection of a combination of midazolam (7.2 mg/kg; Hoffmann La Roche, Basel, Switzerland), fentanyl citrate (0.48 mg/kg; Janssen Pharmaceutica, Beerse, Belgium), and fluanisone (14.8 mg/kg; Janssen). In each animal, the areas of intra-abdominal and unilateral subcutaneous fat content were measured with freehand regions of interest in one slice at the level of the urinary bladder and in a second slice 6 mm above the first one. Fat content was determined as area in square centimeters; total fat content was calculated as mean of the two measurements × 9 mm, i.e., as fat volume in cubic centimeters.

Statistics. Means ± SE are shown. Statistical analyses were performed with the SPSS for Windows system. Statistical comparisons for the differences between mice on high-fat and control diet with regard to body weight and baseline plasma levels of leptin, insulin, and glucose were performed using Student's unpaired *t*-test. Statistical comparisons before and after the fasting were performed with Student's paired *t*-test. Analysis of the normal distribution was performed with the Kolmogorov-Smirnov goodness-of-fit test. Pearson's product-moment correlation was used to estimate linear relationships betwen variables. To describe relationships between two variables while adjusting for the effects of

Table 1. *Recovery of leptin in mouse serum*

Leptin, ng/ml	Concn, ng/ml		Recovery, %
	Observed	Expected	
0	0.4	0.4	100
1	1.3	1.4	93
2	2.2	2.4	92
5	5.1	5.4	94

Leptin at different concentrations was added to a mouse serum pool with a serum leptin concentration of 0.4 ng/ml. Serum was then determined for leptin concentrations and recovery was calculated.

Table 2. *Serum dilution of linearity of mouse leptin radioimmunoassay*

Sample	Volume Sampled, μl	Concentration, ng/ml		Observed in % of Expected Concentration
		Observed	Expected	
A	100	10.5	10.5	100
	75	11.9		113
	50	13.1		125
B	100	8.1	8.1	100
	75	8.7		108
	50	9.1		113
C	100	4.6	4.6	100
	75	4.8		104
	50	5.1		110
D	100	2.2	2.2	100
	75	2.4		107
	50	2.5		113

Serums with known concentrations of leptin were diluted and leptin concentrations of the diluted serums were determined.

Table 3. *Variation within and between assay for radioimmunoassay of leptin*

Sample	Mean, ng/ml*	CV, %	
		Within assay	Between assay
1	0.4 ± 0.1	11.2	14.6
2	1.3 ± 0.1	8.8	7.7
3	2.2 ± 0.1	4.0	5.9
4	5.4 ± 0.2	4.9	3.3

Values are means ± SD. Repeated analysis for concentrations of leptin of 4 different mouse serum samples was performed for calculation of within- and between-assay variation. CV, coefficient of variation. *From 10 replicates.

a third variable, the partial correlation matrix of Pearson's product-moment correlation was used.

RESULTS

Plasma leptin, insulin, and glucose. Plasma leptin increased with age in C57BL/6J mice on the control diet and was 14.2 ± 1.6 ng/ml (n = 15) vs. 5.3 ± 0.6 ng/ml at 2 mo (n = 23, $P < 0.001$; Fig. 1). Consumption of a high-fat diet further increased plasma leptin, because after 10 mo, i.e., at 11 mo of age, plasma leptin had increased to 18.5 ± 1.5 ng/ml in mice on high-fat diet (n = 18, $P = 0.048$ vs. controls; Fig. 1). The high-fat diet also increased plasma insulin and glucose levels, which was evident after 1 mo ($P < 0.001$), whereas body weight was significantly different between the two groups of mice after 6 mo on high-fat diet (Fig. 1).

Correlation with intra-abdominal and subcutaneous fat content. MRI was performed in mice after 10 mo of treatment with high-fat diet (n = 6) or control diet (n = 6). MRI allowed us to visualize the intra-abdominal and subcutaneous fat and illustrated a large difference between the two groups (Fig. 2). Calculating the volume of both intra-abdominal and subcutaneous fat revealed a marked elevation in the group given high-fat diet vs. the group given control diet. Thus intra-abdominal fat content at the level of determination was 0.99 ± 0.087 cm^3 in controls vs. 2.55 ± 0.17 cm^3 in mice subjected to treatment with high-fat diet ($P < 0.001$), and the corresponding figures for subcutaneous fat were 0.32 ± 0.029 vs. 0.77 ± 0.041 cm^3 ($P < 0.001$). Furthermore, both the intra-abdominal (r = 0.92, $P <$ 0.001) and subcutaneous fat volume (r = 0.88, $P <$ 0.001) correlated significantly with log plasma leptin (Fig. 2). These correlation coefficients were similar to those between log plasma leptin and body weight (r = 0.88, $P < 0.001$) and higher than those between plasma insulin and intra-abdominal fat content (r = 0.55, P = 0.062), subcutaneous fat content (r = 0.62, P = 0.031), or body weight (r = 0.64, P = 0.024).

Correlations between plasma leptin and insulin, glucose, body weight, and age. Plasma leptin levels across all animals at 2–11 mo of age (n = 160) correlated with body weight (r = 0.84, $P < 0.001$) as well as plasma insulin (r = 0.33, $P < 0.001$; Fig. 3). The plasma leptin levels did not, however, display a normal distribution (P = 0.0008 in the Kolmogorov-Smirnov goodness-of-fit test), and therefore, in the following correlations, the logarithmic value for plasma leptin was used. Log plasma leptin correlated significantly with body weight when calculated in both groups of mice at all ages (r = 0.68, $P < 0.001$). Also plasma insulin (r = 0.23, P = 0.003) and glucose (r = 0.17, P = 0.035) correlated with body weight, but to lower degrees. Log plasma leptin also correlated significantly with plasma insulin (r = 0.38, $P < 0.001$) and with plasma glucose (r = 0.16, P = 0.041). Partial correlation analysis revealed that log plasma leptin correlated with plasma insulin also after controlling for body weight, i.e., independently of body weight (r = 0.31, $P < 0.001$). We also calculated whether the increase in plasma leptin levels with age is due to the increased body weight by age. Partial correlation analysis between log plasma leptin levels and age in months after controlling for body weight was therefore performed. It was found that, after control for

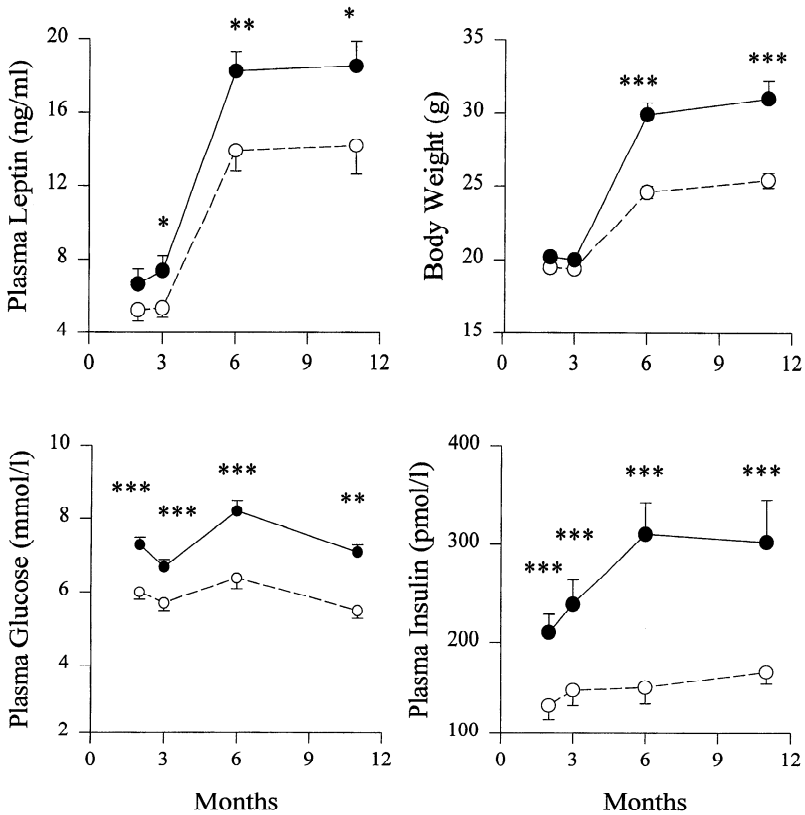

Fig. 1. Plasma levels of leptin, glucose, and insulin and body weight in C57BL/6J mice at various ages between 2 and 11 mo. Mice were given normal (○) or high-fat (●) diet for 1, 2, 6, or 10 mo. Means ± SE are shown. Probability level of random difference between groups: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. There were 15–35 animals in each group.

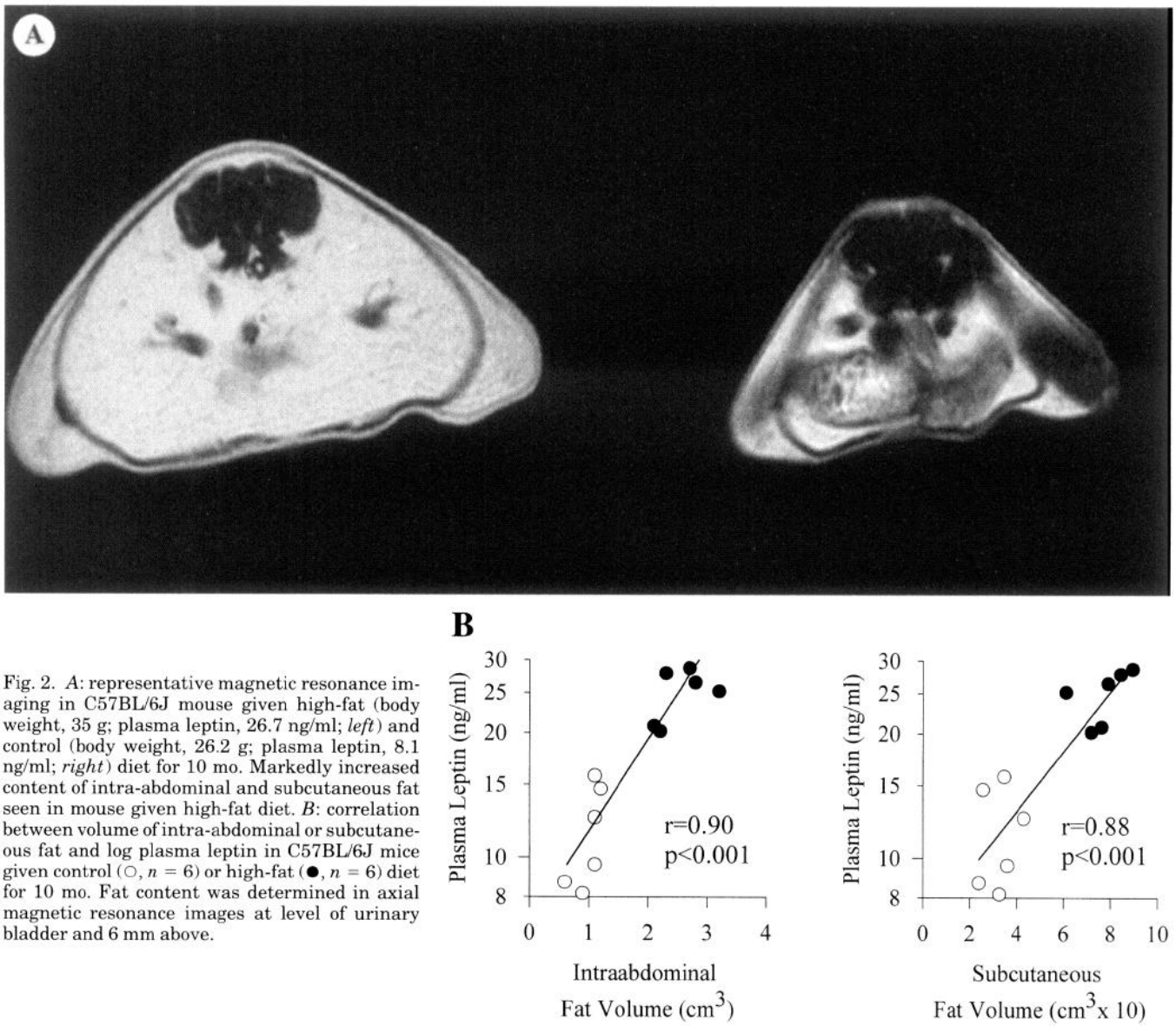

Fig. 2. *A*: representative magnetic resonance imaging in C57BL/6J mouse given high-fat (body weight, 35 g; plasma leptin, 26.7 ng/ml; *left*) and control (body weight, 26.2 g; plasma leptin, 8.1 ng/ml; *right*) diet for 10 mo. Markedly increased content of intra-abdominal and subcutaneous fat seen in mouse given high-fat diet. *B*: correlation between volume of intra-abdominal or subcutaneous fat and log plasma leptin in C57BL/6J mice given control (○, $n = 6$) or high-fat (●, $n = 6$) diet for 10 mo. Fat content was determined in axial magnetic resonance images at level of urinary bladder and 6 mm above.

body weight, log plasma correlated significantly with age in control mice ($r = 0.25$, $P = 0.031$) but not in mice given the high-fat diet ($P = 0.69$).

Plasma leptin after 48-h fasting. After 2 mo of treatment with high-fat diet or control diet, mice underwent a 48-h fasting period. Figure 4 shows that plasma levels of leptin, insulin, and glucose as well as body weight decreased significantly during fasting in both groups. Comparing the percent reduction from values before fasting revealed that mice on control diet had a more marked reduction in plasma leptin ($-44 \pm 3\%$) than those on high-fat diet ($-16 \pm 7\%$, $P = 0.001$). Similarly, the reduction in body weight was higher in control mice (-3.2 ± 0.1 g) than in high-fat diet-treated mice (-2.8 g $\pm$ 0.1 g, $P = 0.002$), whereas the reduction in plasma insulin was the same in the two groups of mice [-102 ± 20 pmol/l in control mice vs. -104 ± 35 pmol/l in high-fat diet-treated mice, nonsignificant (NS)]. Thus the difference in plasma insulin between the two groups of mice persisted unaltered throughout the fasting period. In contrast, the reduction in plasma glucose was more pronounced in high-fat diet-treated mice (-2.9 ± 0.2 mmol/l) than in control mice (-2.0 ± 0.3 mmol/l, $P = 0.020$). Correlation analysis revealed that the reduction in plasma leptin during fasting correlated with the reduction in plasma insulin ($r = 0.43$, $P = 0.012$; Fig. 5). In contrast, the reduction in leptin during fasting did not correlate with prefasting body weight ($r = 0.12$, NS) or with the change in body weight during fasting ($r = 0.23$, NS).

DISCUSSION

In this study, we have developed a new radioimmunoassay for measuring plasma leptin in mice, thus allowing investigation of factors involved in the regulation of plasma leptin in rodents. The radioimmunoassay was found to be specific, exhibiting a high recovery (>90%) and low intra- and interassay variability. With this new radioimmunoassay, we examined the regulation of

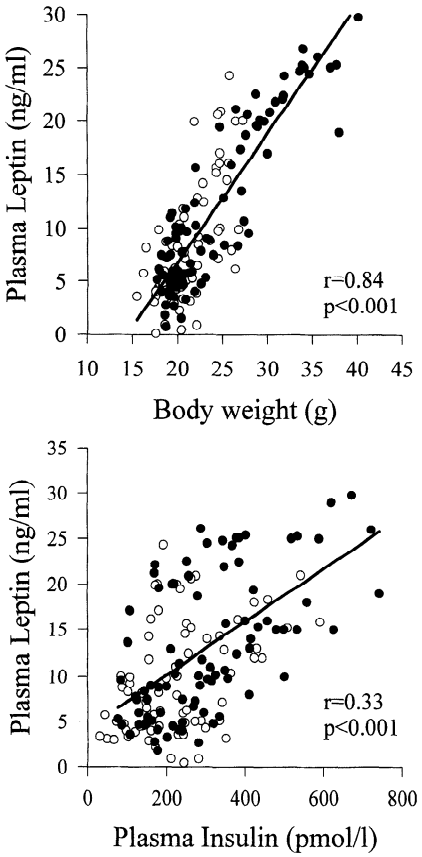

Fig. 3. Correlation of plasma leptin and body weight and plasma leptin and plasma insulin in C57BL/6J mice of 2, 3, 7, or 11 mo of age given control (○, n = 73) or high-fat (●, n = 87) diet for 1, 2, 6, or 10 mo (total n = 160). Regression line and P and r values for correlation are also shown.

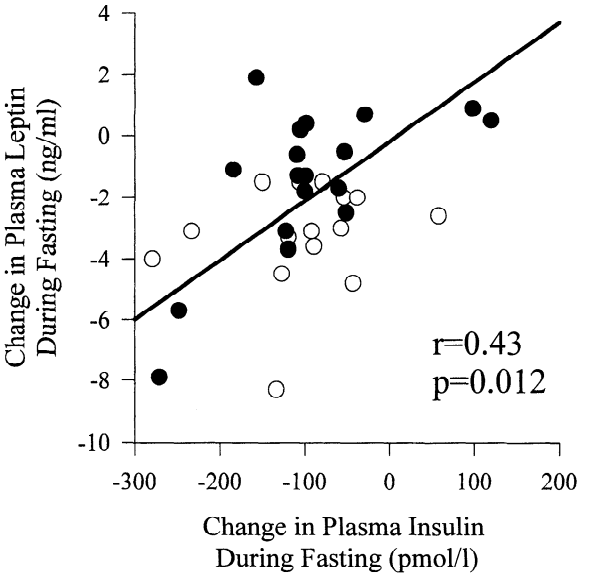

Fig. 5. Correlation of changes in plasma insulin and changes in plasma leptin during a 48-h fast in 3 mo-old C57BL/6J mice treated with a high-fat (●, n = 18) or control (○, n = 15) diet for 2 mo. Regression line and P and r values for correlation are also shown.

plasma leptin under conditions when plasma insulin is increased (during insulin resistance) and reduced (during fasting) in C57BL/6J mice. We demonstrate that plasma leptin increases with age in the mice and that challenging the animals with a high-fat diet further augments this increase in plasma leptin. These results are in agreement with those of previous studies showing increased *ob* gene expression in adipocytes of C57BL/6J mice after high-fat diet for 14 or 16 wk (6, 23)

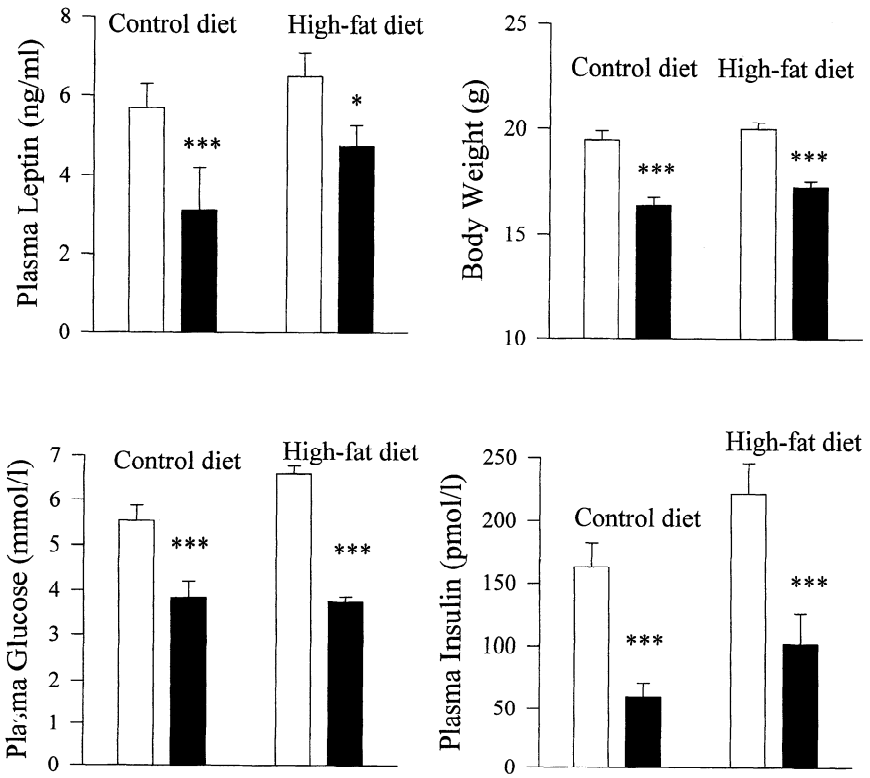

Fig. 4. Plasma levels of leptin, glucose, and insulin and body weight in C57BL/6J mice at age 3 mo given a control (n = 15) or high-fat (n = 18) diet for 2 mo, before (open bars) and after (filled bars) 48-h fasting. Means ± SE are shown. $^{*}P < 0.05$; $^{***}P < 0.001$.

and increased circulating leptin as determined by quantitative Western blot analysis in FVB mice after 12 wk on high-fat diet (10). We also found a correlation between plasma leptin and body weight in accordance with previous reports (10, 22). The age-dependent increase in leptin levels appears to be largely dependent on the increased body weight, although we also found an independent influence of age on plasma leptin. Hence this study shows that body weight, age, and fat content of the diet all affect plasma leptin concentrations in mice. In this study, we also assessed the body fat content using MRI, which is a sensitive technique for distinguishing fat from surrounding tissue (1). We found that both intra-abdominal and subcutaneous fat content increased in mice on the high-fat diet and that plasma leptin correlated to a high degree ($r > 0.9$) with fat content. These data suggest that leptin is synthesized and secreted from the fat cell in relation to body fat content.

Challenging C57BL/6J mice with a high-fat diet has previously been shown to induce insulin resistance (29). This is apparent in the present study, because hyperinsulinemia was observed after 1 mo of treatment with high-fat diet. We found that plasma leptin correlates with plasma insulin in mice ($r = 0.38$). A previous study showed that *ob* gene expression correlates with plasma insulin in C57BL/6J mice (24), and we have recently found correlations between plasma leptin and circulating insulin in humans (16, 19). This correlation between circulating levels of insulin and leptin might be secondary to a stimulatory influence of insulin on *ob* gene expression. However, possible influences of leptin on insulin secretion, as inferred from a recent demonstration of leptin receptors on the insulin-producing β-cells (17), cannot be excluded.

Although not previously demonstrated, our finding of a significant correlation between plasma leptin and plasma insulin in mice might be regarded as expected, because body weight correlates with both plasma leptin and plasma insulin. To examine whether the correlation between plasma leptin and plasma insulin is simply due to both parameters correlating with body weight, we performed a partial correlation analysis between plasma leptin and plasma insulin, controlling for the influence of body weight. This analysis revealed that plasma leptin and plasma insulin were correlated independently of body weight. Therefore, the present study suggests that leptin and insulin are either related to each other through a mutual action or a third factor is involved in the regulation of plasma leptin and plasma insulin. One explanation could be that insulin stimulates the synthesis and secretion of leptin, because increased fat content is associated with insulin resistance and hyperinsulinemia. Such a hypothesis is supported by several reports that insulin stimulates *ob* gene expresion in adipocytes (8, 18, 24, 27) and that supraphysiological insulin infusions increase plasma leptin in humans within 4–6 h (15, 30). Alternatively, the increased plasma leptin levels may be related to the insulin resistance and therefore have evolved independently of hyperinsulinemia.

Fasting was found to reduce plasma leptin levels coincidentally with the reduced body weight and lowering of insulin and glucose. Previous studies have shown that fasting reduces the *ob* gene expression in both rats and mice (3, 11, 21, 24, 28), and it has also been shown that fasting reduces circulating leptin in mice (2, 11) and rats (13). Leptin might therefore be a hormonal signal of nutritional status, both in obesity, when plasma leptin is increased, and in fasting, when plasma leptin is reduced. In fasting, the reduction in plasma leptin may contribute to the increased drive for food intake. Interestingly, we found that the reduction in plasma leptin during fasting was impaired in mice made obese and insulin-resistant on a high-fat diet despite these animals having an exaggerated reduction of plasma glucose. One mechanism underlying this insensitivity would be the insulin resistance in these mice, if it is the reduced insulin level that contributes to the lowered leptin secretion during fasting. This is a possible explanation, because we found that the reduction in plasma leptin during fasting correlated with the reduction in circulating insulin levels and not with prefasting body weight or with changes in body weight. It was previously shown that administering leptin to fasted mice prevented the starvation-induced effects on the gonadal, adrenal, and thyroid axes without influencing the lowered insulin and glucose levels (2). This was suggested to indicate that insulin and leptin may cooperate to regulate the neuroendocrine responses to starvation. Our present results also suggest that insulin seems to be involved in the regulation of circulating leptin during starvation in mice.

In summary, the results of this study show that plasma leptin is increased with age and after a high-fat diet in C57BL/6J mice and that plasma leptin concentrations correlate with body weight and with intra-abdominal and subcutaneous fat content. In addition, plasma leptin correlates with plasma insulin independently of body weight. Furthermore, 48-h fasting reduces plasma leptin in conjunction with reduced body weight and plasma insulin. The fasting-induced reduction in plasma leptin is inhibited by high-fat diet and correlates with the reduction in plasma insulin. We conclude that plasma leptin concentrations in mice are increased with age and affected by nutritional status and that plasma leptin is related to plasma insulin independently of adiposity.

Perspectives

Leptin, a protein produced in and released from adipocytes, was recently discovered by positional cloning of the *ob* gene in obese diabetic mice. Acting via cytokine-like receptors in the central nervous system, leptin reduces food intake and body weight in mice. Leptin therefore appears to be a hormonal signal in a feedback system controlling body weight. Thus factors regulating food intake and body weight might operate through changes in circulating leptin. It is therefore important to characterize the regulation of circulating leptin. Most importantly, circulating leptin is controlled by the body fat content. However, adiposity

explains only ~50% of the variations in plasma leptin, suggesting that other factors are involved in the physiological regulation of circulating leptin. Previous studies have demonstrated that circulating leptin is higher in women than in men, undergoes a diurnal variation, and appears to be related to insulin secretion. With a newly developed radioimmunoassay for murine leptin, we found that a high-fat diet increases and fasting reduces circulating leptin in mice. These results support the hypothesis that leptin is a hormonal signal of nutritional status, both in obesity, when circulating leptin is increased, and in fasting, when circulating leptin is reduced. The demonstration in the present study that leptin correlates with circulating insulin independently of body weight indicates that insulin is involved in the regulation of circulating leptin in mice. Further studies are now needed to examine the mechanisms regulating leptin secretion and the physiological implications of the relationship between insulin and leptin.

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